Data Article

Metabarcoding data of mitochondrial cytochrome c oxidase subunit 1 gene from bulk community of aquatic organisms collected from Nara Prefecture, Japan

Kei Wakimura a, Koji Inai a, Kazumi Tanida c, Kozo Watanabe d, Mikio Kato a,b,*

a Faculty of Liberal Arts and Sciences, Osaka Metropolitan University - Nakamozu Campus, 1-1 Gakuencho, Sakai 599-8531, Japan
b Department of Biology, Graduate School of Science, Osaka Metropolitan University - Nakamozu Campus, 1-1 Gakuencho, Sakai 599-8531, Japan
c Osaka Museum of Natural History, Nagai Park, Higashi-sumiyoshi, Osaka, 546-0034, Japan
d Center for Marine Environment Studies, Ehime University, Bunkyo-cho 3, Matsuyama 790-8577, Japan

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ABSTRACT

Riverine metabarcoding data were obtained from the Takamigawa River, a tributary of the Kinokawa River, in Nara Prefecture (Central Honshu, Japan). We extracted DNA from bulk community samples of aquatic organisms, most of which could not be morphologically identified at species level due to their small body size (0.12 - 2 mm length). A partial coding region of the mitochondrial cytochrome c oxidase subunit 1 gene (cox1) was amplified using PCR, and the amplicon was subjected to high-throughput parallel sequencing (Illumina MiSeq). The 313 bp paired-end sequence reads were classified into operational taxonomic units (OTUs), their species boundaries were delineated using the Generalised Mixed Yule Coalescent (GMYC) method, and taxonomic names of the GMYC species were assigned using basic local alignment search tool (BLAST) against International DNA Databases (INSD: GenBank, ENA, and DDBJ).

* Corresponding author.
E-mail address: mkato@omu.ac.jp (M. Kato).

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Specifications Table

| Subject:                                      | Biodiversity                                           |
|----------------------------------------------|--------------------------------------------------------|
| Specific subject area:                       | DNA taxonomy and species identification of stream insects |
| Type of data:                                | Figure, Table, and amplicon sequence data              |
| Data acquisition:                           | Illumina MiSeq platform, MrBayes 3.2.7a, SPLITS package in R |
| Data format:                                 | Raw sequence reads                                     |
| Description of data collection:             | Analysed metabarcoding data                            |
|                                             | DNA extraction of three bulk community samples of aquatic organisms, amplicon sequencing of cox1, quality-filtering of sequence reads, operational taxonomic units (OTUs) clustering of sequence reads using ad hoc R script, building a phylogenetic tree of OTUs using Markov chain Monte Carlo (MCMC) by MrBayes 3.2.7a with the GTR+I+G molecular clock model, estimation of taxonomic boundaries among OTUs based on GMYC approach using SPLITS package in R, and BLAST search against INSD. |
| Data source location:                       | Bulk community samples of aquatic organisms collected from Takamigawa River, Nara Prefecture, Central Honshu, Japan. |
|                                             | Sampling site: Aridoshi (ARD), 34°23′23″N 135°59′17″E |
|                                             | Sampling site: Shisetsu at Kozugawa (SST), 34°23′53″N 135°59′34″E |
| Data accessibility:                         | With the article: Phylogenetic tree, taxonomy, abundance of the OTUs. In a public repository: DNA Databank of Japan (DDBJ) and Mendeley Data. The sequencing data are available in DDBJ under the SRA ID: DRA012522; BioProject No. PRJDB11912; BioSample: SAMD00388525, SAMD00388526, SAMD00388527, SAMD00388528, SAMD00388529, SAMD00388530, SAMD00388531, SAMD00388532; Run: DRR311861, DRR311862, DRR311863, DRR311864, DRR311865, DRR311866, DRR311867, DRR311868. OTU sequences in FASTA format and an R script for clustering OTUs are deposited in Mendeley Data with DOI: 10.17632/phf4kt37s.1 |

Value of the Data

- The metabarcoding result enables us to evaluate the efficacy of DNA-based biodiversity assessment in comparison with conventional morphology-based species identification data collected from the same localities.
- The new OTU clustering algorithm that compare the read abundances between neighbouring OTUs is available as an R script, which could be used widely to process amplicon sequence data.
- The results are beneficial for researchers working on both DNA metabarcoding and conventional morphology-based faunal surveys.
- The data will serve as a reference barcode sequence data of benthic fauna in mountain streams in the Central Honshu, Japan.

1. Data Description

Raw sequence reads of partial coding regions of cox1 in riverine metagenomes are deposited in DNA Data Bank of Japan (DDBJ). DDBJ accession numbers DRR311861 and DRR311862 were obtained from the run environment (slow riffle) of Aridoshi (ARD), DRR311863-DRR311865 from the rapid environment of Shisetsu (SST), and DDR311866-DRR311868 from the run environment of Shisetsu (SST). There were 472,697 reads in total of DRR311861- DRR311868, and they were classified into 888 OTUs.
Phylogenetic tree of the 888 OTUs was reconstructed by MrBayes 3.2.7a based on the GTR+I+G molecular clock model, and the species boundaries were delineated using the Generalised Mixed Yule Coalescent (GMYC) method (Fig. 1). A total of 369 GMYC species were presumed from 888 OTUs.

Representative sequences of the GMYC species (the most abundant OTUs in the species clusters; marked with GMYC IDs in Fig. 1) were queried on INSD, and the sequence data showing the highest similarity were retrieved (Table 1). According to the critical similarity scores for species identification [1], OTUs showing similarity scores (percent identity) greater than 95% are considered to be properly deduced. In the present results, 137 out of 369 GMYC species were assigned to INSD with >95% similarity. The DNA barcode references of the remaining unassigned GMYC species may not have been registered in INSD yet. These unassigned GMYC species can be extrapolated to coarser level taxonomic names (i.e., genus, family, or order) according to the critical similarity scores proposed by Inai et al. [1].

2. Experimental Design, Materials and Methods

2.1. Collection of specimens

Benthic organisms were collected using a hand net (mesh size: 200 per inch) in a perturbed riverbed by kicking at two sampling stations: ARD (on 17 March 2017) and SST (on 29 June 2017). Sampling was performed in the run environment (slow riffle) of both stations ARD and SST, and in rapid environment of SST (three community samples in total). The specimens were immersed in 70% ethanol until DNA was isolated. DNA of the three bulk community samples (one from ARD and two from SST) were separately extracted using conventional proteinase K treatment/phenol extraction/ethanol precipitation, as previously described [3].

2.2. DNA sequencing

The DNA libraries for sequencing were constructed by 2-step tailed PCR. The first PCR amplifications of the target gene (partial coding region of cox1) were performed using PrimeSTAR® HS DNA Polymerase (Takara Bio Co., Ltd., Kusatsu, Japan) for 35 cycles of 98°C-10 s / 40°C-5 s / 72°C-60 s with an initial incubation at 95°C for 1 min and a final elongation at 72°C for 7 min, using a set of primers IntF and HCOM [2]. The nucleotide sequences of the primers (containing the linker for the second PCR) were as follows:

IntF, ACACCTTTCTCCCTACACGCGCCTTCCGATCTGGWACWGWTGAAACWGTWTAYCCYCC;
HCOM, GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAHACTTCNNGGGTGKCCRAARAATCA.

The amplified fragments were purified by AMPure XP (Beckman Coulter, Brea, CA, USA). The second PCR was performed using ExTaQ® DNA Polymerase (Takara Bio Co. Ltd.) for 12 cycles of 94°C-30 s / 60°C-30 s / 72°C-30 s with an initial incubation at 94°C for 2 min and a final elongation at 72°C for 5 min, with a set of DNA primers containing octanucleotide tag for indexing. The nucleotide sequences of primers were as follows:

2nd-F, AATGATACGGCCAGCAGCTACGTACCAGT-3[octanucleotide tag]-ACACTCTTTCCCTACACGC;
2nd-R, CAACAGAAAGACCGCATACTACAGATTAGCTGCAGTGACTGGAGTCCAGACGTTG.

Two technical replicates for ARD and three technical replicates for each of two SST samples were prepared with different tags. The tag sequences for DRR311861, DRR311862, DRR311863, DRR311864, DRR311865, DRR311866, DRR311867, and DRR311868 were TCGACTAG, TTCTAGCT, CCTAGAGT, GCGTAAG, CTATTAAG, AAGGCTAT, GAGCCTTA, and TTATGCGA, respectively. Quality of
Table 1
List of INSD data showing highest similarity with representative OTUs assigned as species by GMYC.

| GMYC ID | (Number of identical sites/query size) | Similarity scores (% identity) | INSD accession number | Organism name | Family | Order | Remark on taxon |
|---------|---------------------------------------|--------------------------------|-----------------------|---------------|--------|-------|----------------|---|
| 10      | (313/313)                             | 100.0                          | MK774296              | Paracingymula zhiltzovae / Heptageniidae / Ephemeroptera |        |       |                 |
| 11      | (313/313)                             | 100.0                          | KP970686              | Epeorus aesculus / Heptageniidae / Ephemeroptera           |        |       |                 |
| 12      | (313/313)                             | 100.0                          | MK774388              | Epeorus curvatulus / Heptageniidae / Ephemeroptera          |        |       |                 |
| 15      | (313/313)                             | 100.0                          | JQ655115              | Epeorus latifolium / Heptageniidae / Ephemeroptera          |        |       |                 |
| 17      | (313/313)                             | 100.0                          | MK774354              | Epeorus latifolium / Heptageniidae / Ephemeroptera          |        |       |                 |
| 18      | (313/313)                             | 100.0                          | LC513137              | Afronurus yoshidae / Heptageniidae / Ephemeroptera          |        |       | Ecdyonurus yoshidae |
| 20      | (313/313)                             | 100.0                          | LC106815              | Isonychia japonica / Isonychiidae / Ephemeroptera           |        |       | Takahashi, 1924 |
| 22      | (313/313)                             | 100.0                          | GU354162              | Cinygymula sp. MK-2010 / Heptageniidae / Ephemeroptera      |        |       |                 |
| 25      | (313/313)                             | 100.0                          | MK774360              | Drunella trispina / Ephemereillidae / Ephemeroptera         |        |       |                 |
| 27      | (313/313)                             | 100.0                          | MK774334              | Drunella ishiyamana / Ephemereillidae / Ephemeroptera      |        |       |                 |
| 34      | (313/313)                             | 100.0                          | LC489696              | Dipteromimus tipuliformis / Dipteromimidae / Ephemeroptera |        |       |                 |
| 38      | (313/313)                             | 100.0                          | GU354161              | Paraleptophlebia chocolata / Leptophlebiidae / Ephemeroptera |        |       |                 |
| 39      | (313/313)                             | 100.0                          | MN961294              | Ephemer a strigata / Ephemereda / Ephemeroptera            |        |       |                 |
| 41      | (313/313)                             | 100.0                          | KP970715              | Ephemereillidae sp. OPU_BS_E2014-4 / Ephemereillidae / Ephemeroptera |        |       |                 |
| 66      | (313/313)                             | 100.0                          | MN344567              | Kamimuria tibialis / Perlidae / Plecoptera                 |        |       |                 |
| 70      | (313/313)                             | 100.0                          | MN961334              | Neoperla sp. OPU BS 2019-094-DB-PL / Perlidae / Plecoptera |        |       |                 |
| 74      | (313/313)                             | 100.0                          | MK774376              | Paragnetina sp. OPU_BS_2017-320-YS-PL / Perlidae / Plecoptera |        |       |                 |
| 76      | (313/313)                             | 100.0                          | AB770094              | Togoperla limbata / Perlidae / Plecoptera                  |        |       |                 |
| 77      | (313/313)                             | 100.0                          | MK774375              | Paragnetina sp. OPU_BS_2017-319-YS-PL / Perlidae / Plecoptera |        |       |                 |
| 81      | (313/313)                             | 100.0                          | MK492252              | Perloymia isobae / Leuctriidae / Plecoptera                 |        |       |                 |
| 91      | (313/313)                             | 100.0                          | LC462303              | Corynoneura celtica / Chironomidae / Diptera               |        |       |                 |
| 96      | (313/313)                             | 100.0                          | LC462341              | Cricotopus metatibialis / Chironomidae / Diptera           |        |       |                 |
| 97      | (313/313)                             | 100.0                          | LC462339              | Cricotopus metatibialis / Chironomidae / Diptera           |        |       |                 |
| 119     | (313/313)                             | 100.0                          | AB838600              | Cricotopus bimaculatus / Chironomidae / Diptera            |        |       |                 |
| 126     | (313/313)                             | 100.0                          | LC462328              | Synorthocladius tanaparvulus / Chironomidae / Diptera       |        |       |                 |
| 137     | (313/313)                             | 100.0                          | LC329260              | Rheopelopia joganflava / Chironomidae / Diptera            |        |       |                 |
| 161     | (313/313)                             | 100.0                          | LC329063              | Cladotanytarsus vanderwulpi / Chironomidae / Diptera       |        |       |                 |
| 171     | (313/313)                             | 100.0                          | LC342229              | Tanytarsus tamaundecimus / Chironomidae / Diptera          |        |       |                 |
| 177     | (313/313)                             | 100.0                          | LC329303              | Tanytarsus arduennensis / Chironomidae / Diptera           |        |       |                 |
| 183     | (313/313)                             | 100.0                          | LC462285              | Microtendipes famiefeus / Chironomidae / Diptera           |        |       |                 |
| 185     | (313/313)                             | 100.0                          | LC329175              | Polypedilum asakawaense / Chironomidae / Diptera           |        |       |                 |
| 199     | (313/313)                             | 100.0                          | JQ655116              | Baetis sp. OPU_BS_B2011-494 / Baetidae / Ephemeroptera     |        |       |                 |

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Table 1 (continued)

| GMYC ID | (Number of identical sites/ query size) | Similarity scores (% identity) | INSD accession number | Organism name/ Family/ Order | Remark on taxon |
|---------|----------------------------------------|-------------------------------|-----------------------|------------------------------|----------------|
| 212     | (313/313)                              | 100.0                         | KPS970716             | Baetidae/ Ephemeroptera      | Paracyclops auslans/ Coleoptera |
| 213     | (313/313)                              | 100.0                         | KF563015              | Baetidae/ Ephemeroptera      | Paracyclops auslans/ Coleoptera |
| 215     | (313/313)                              | 100.0                         | MH260764              | Stenopsyche sauteri/ Trichoptera |
| 216     | (313/313)                              | 100.0                         | KX291699              | Stenopsyche marmorata/ Trichoptera |
| 225     | (313/313)                              | 100.0                         | JX448423              | Curculionidae/ Coleoptera |
| 230     | (313/313)                              | 100.0                         | KX106254              | Rhyacophila kawamurae/ Rhyacophila/ Trichoptera |
| 231     | (313/313)                              | 100.0                         | MK261430              | Rhyacophila kisoensis/ Rhyacophila/ Trichoptera |
| 237     | (313/313)                              | 100.0                         | KX104086              | Goera japonica/ Goeridae/ Trichoptera |
| 238     | (313/313)                              | 100.0                         | KX104891              | Goera squamifera/ Goeridae/ Trichoptera |
| 240     | (313/313)                              | 100.0                         | HQ967404              | Trichoptera sp. BOLD:AAN9649/ Trichoptera |
| 246     | (313/313)                              | 100.0                         | LT903836              | Nais communis/ Naididae/ Haplotoxida |
| 255     | (313/313)                              | 100.0                         | KY284174              | Eiseniella tetraedra/ Lumbricidae/ Crassiclitellata |
| 295     | (313/313)                              | 100.0                         | MH260783              | Cheumatopsyche infascia/ Hydropsychidae/ Trichoptera |
| 296     | (313/313)                              | 100.0                         | MH260789              | Cheumatopsyche galloisi/ Hydropsychidae/ Trichoptera |
| 297     | (313/313)                              | 100.0                         | KX104427              | Ceratopsyche orientalis/ Hydropsychidae/ Trichoptera |
| 298     | (313/313)                              | 100.0                         | MK774339              | Ceratopsyche setensis/ Hydropsychidae/ Trichoptera |
| 302     | (312/312)                              | 100.0                         | HQ967450              | Trichoptera sp. BOLD:AAN9228/ Trichoptera |
| 305     | (313/313)                              | 100.0                         | AP010696              | Homo sapiens/ Hominidae/ Primates |
| 306     | (295/295)                              | 100.0                         | AB988797              | Rhinogobius flumineus/ Gobiidae/ Gobiiformes |
| 307     | (301/301)                              | 100.0                         | AB708313              | Lestes temporalis/ Lestidae/ Odonata |
| 308     | (301/301)                              | 100.0                         | AB708713              | Oxychomphalus viridicostus/ Gomphidae/ Odonata |
| 322     | (313/313)                              | 100.0                         | GU722900              | Hydra vulgaris/ Hyridae/ Anthoathecata |
| 337     | (313/313)                              | 100.0                         | MF000493              | Craspedacusta sowerbii/ Oliniidae/ Limnomedusae |
| 351     | (313/313)                              | 100.0                         | JN574872              | Calonectica colhounii/ Nectriaceae/ Hypocreales |
| 354     | (313/313)                              | 100.0                         | MT010913              | Fusarium globoSUM/ Nectriaceae/ Hypocreales |
| 3       | (312/313)                              | 99.7                          | MK774315              | Rhitrogena japonica/ Heptagenidae/ Ephemeroptera |
| 56      | (312/313)                              | 99.7                          | MK774371              | Oyamia sp. OPU_BS_2017-315-YS-PL/ Perlidae/ Plecoptera |
| 79      | (312/313)                              | 99.7                          | MK132349              | Amphinemura megaloba/ Nemouridae/ Plecoptera |
| 98      | (312/313)                              | 99.7                          | LC050960              | Cricotopus metatibialis/ Chironomidae/ Diptera |
| 135     | (312/313)                              | 99.7                          | LC329252              | Rheopelopia joganflava/ Chironomidae/ Diptera |

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| GMYC ID | Number of identical sites/query size | Similarity scores (% identity) | INSD Accession number | Organism name/Family/Order | Remark on taxon |
|---------|-------------------------------------|---------------------------------|------------------------|-----------------------------|-----------------|
| 136     | (312/313)                           | 99.7                            | LC329258               | Rheopelopia joganflava / Chironomidae / Diptera |
| 166     | (312/313)                           | 99.7                            | EF585416               | Stempellinella coronata / Chironomidae / Diptera |
| 182     | (312/313)                           | 99.7                            | LC329124               | Microtendipes britteni / Chironomidae / Diptera |
| 188     | (312/313)                           | 99.7                            | LC329243               | Polypedilum unifascium / Chironomidae / Diptera |
| 223     | (312/313)                           | 99.7                            | LT991411               | Ochthebius japonicus / Hydraenidae / Coleoptera |
| 235     | (312/313)                           | 99.7                            | MN961310               | Glossosoma ussuricum / Glossosomatidae / Trichoptera |
| 336     | (312/313)                           | 99.7                            | MF177710               | Crasedacusta sowerbii / Olindiidae / Limnomedusae | peach blossom jellyfish |
| 1       | (311/313)                           | 99.4                            | MK774405               | Rhithrogena japonica / Heptageniidae / Ephemeroptera |
| 8       | (311/313)                           | 99.4                            | MK774358               | Rhithrogena tetrapunctigera / Heptageniidae / Ephemeroptera |
| 9       | (311/313)                           | 99.4                            | MN961320               | Heptageniidae sp. OPU BS 2019-040-DB-EP / Heptageniidae / Ephemeroptera |
| 26      | (311/313)                           | 99.4                            | MK774398               | Drunella kohnoi / Ephemereellidae / Ephemeroptera |
| 107     | (311/313)                           | 99.4                            | LC329151               | Orthocladius kanii / Chironomidae / Diptera |
| 127     | (311/313)                           | 99.4                            | LC329155               | Parakiefferiella tamariangulata / Chironomidae / Diptera |
| 129     | (311/313)                           | 99.4                            | LC329157               | Parametricnemus stylatus / Chironomidae / Diptera |
| 179     | (311/313)                           | 99.4                            | LC329188               | Polypedilum hiroshimaense / Chironomidae / Diptera |
| 194     | (311/313)                           | 99.4                            | MN961306               | Apsilochorema sutshanum / Hydrobiosidae / Trichoptera |
| 196     | (311/313)                           | 99.4                            | MK144522               | Nymphomyia alba / Nymphomyiidae / Diptera |
| 197     | (311/313)                           | 99.4                            | KP970694               | Baetis sp. MK-2015d / Baetidae / Ephemeroptera |
| 234     | (311/313)                           | 99.4                            | MN961311               | Glossosoma altaicum / Glossosomatidae / Trichoptera |
| 301     | (311/313)                           | 99.4                            | HQ967424               | Trichoptera sp. BOLD:AAN3967 / / Trichoptera |
| 21      | (310/313)                           | 99.0                            | MK774393               | Epeorus ikanonis / Heptageniidae / Ephemeroptera |
| 55      | (310/313)                           | 99.0                            | MK774343               | Baetidae sp. OPU BS 2017-141-MA-EP / Baetidae / Ephemeroptera |
| 123     | (310/313)                           | 99.0                            | LC462337               | Orthocladius tamarutilus / Chironomidae / Diptera |
| 164     | (310/313)                           | 99.0                            | LC329266               | Rhoetanytarsus tamasecondus / Chironomidae / Diptera |
| 168     | (310/313)                           | 99.0                            | LC329147               | Neozavrelia tamanona / Chironomidae / Diptera |
| 187     | (310/313)                           | 99.0                            | LC329176               | Polypedilum asprorium / Chironomidae / Diptera |
| 294     | (310/313)                           | 99.0                            | KK103761               | Psychomyia nipponica / Psychomyiidae / Trichoptera |
| 86      | (309/313)                           | 98.7                            | LC462305               | Corynoneura kibunata / Chironomidae / Diptera |
| 95      | (309/313)                           | 98.7                            | LC462311               | Thiemenanniella majuscula / Chironomidae / Diptera |
| 159     | (309/313)                           | 98.7                            | LC329058               | Cladotanytarsus vansewulpi / Chironomidae / Diptera |
| 210     | (309/313)                           | 98.7                            | K9970709               | Acentrella gnom / Baetidae / Ephemeroptera |
| 242     | (309/313)                           | 98.7                            | GQ355370               | Nais variabilis / Naididae / Haplotaxida / Oligochaeta |
| 250     | (309/313)                           | 98.7                            | GQ355366               | Chaetogaster diaphanus / Naididae / Haplotaxida / Oligochaeta |
| 45      | (308/313)                           | 98.4                            | JQ655113               | Serratella setigera / Ephemereellidae / Ephemeroptera |

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Table 1 (continued)

| GMYC ID | (Number of identical sites/query size) | Similarity scores (% identity) | INSD accession number | Organism name/ Family/ Order | Remark on taxon |
|---------|----------------------------------------|---------------------------------|-----------------------|-----------------------------|-----------------|
| 46      | (308/313)                              | 98.4                            | JQ655113 Serratella setigera / Ephemerellidae / Ephemeroptera |
| 85      | (308/313)                              | 98.4                            | LC462302 Corynoneura tenuistyla / Chironomidae / Diptera |
| 90      | (308/313)                              | 98.4                            | LC462308 Corynoneura lobata / Chironomidae / Diptera |
| 113     | (308/313)                              | 98.4                            | LC329246 C / Chironomidae / Diptera |
| 236     | (308/313)                              | 98.4                            | KX106735 Rhacophila angulata / Rhacophilidae / Trichoptera * |
|         |                                        |                                 |                       |                             | species not recorded from Japan |
| 16      | (307/313)                              | 98.1                            | JQ655115 Epeorus latifolium / Heptageniidae / Ephemeroptera |
| 31      | (307/313)                              | 98.1                            | LC481971 Cincticostella elongatula / Ephemerellidae / Ephemeroptera |
| 92      | (307/313)                              | 98.1                            | LC329294 Thienemanniella flaviscutella / Chironomidae / Diptera |
| 103     | (307/313)                              | 98.1                            | LC329301 Tvetenia tamaflava / Chironomidae / Diptera |
| 133     | (307/313)                              | 98.1                            | KY497570 Simulium quinquestriatum / Simuliidae / Diptera |
| 53      | (306/313)                              | 97.8                            | LC462319 Nilotanytarsus dubius / Chironomidae / Diptera |
| 209     | (306/313)                              | 97.8                            | KF563060 Nigrobaetis chororatus / Baetidae / Ephemeroptera |
| 224     | (306/313)                              | 97.8                            | KM376576 Hydrocyphon satoi / Scirtidae / Coleoptera |
| 29      | (305/312)                              | 97.8                            | LC461422 Drunella ishiyamana / Ephemerellidae / Ephemeroptera |
| 19      | (305/313)                              | 97.4                            | MK774389 Edyonurus sp. OPU_B5_2018-038-IN-EP / Heptageniidae / Ephemeroptera |
| 180     | (305/313)                              | 97.4                            | AB731460 Polyplegium takaense / Chironomidae / Diptera |
| 244     | (305/313)                              | 97.4                            | LN810267 Nais brescheri / Naididae / Haplotaxida |
| 299     | (305/313)                              | 97.4                            | KX291233 Ocetis raphava / Leptoceridae / Trichoptera * |
|         |                                        |                                 |                       |                             | Oligochaeta species not recorded from Japan |
| 362     | (304/312)                              | 97.4                            | MK468491 Ascochyla pisi / Didymellaceae / Pleosporales |
| 30      | (304/313)                              | 97.1                            | LC481981 Cincticostella orientalis / Ephemerellidae / Ephemeroptera |
| 118     | (304/313)                              | 97.1                            | KX037940 Tiphobiosis hinewai / Hydrobiosidae / Trichoptera * |
|         |                                        |                                 |                       |                             | species not recorded from Japan |
| 131     | (304/313)                              | 97.1                            | MG551479 Simulium bidentatum / Simuliidae / Diptera |
| 160     | (304/313)                              | 97.1                            | LC329058 Cladotanytarsus vanderwulpi / Chironomidae / Diptera |
| 181     | (304/313)                              | 97.1                            | LC329088 Demicryptochironomus vulneratus / Chironomidae / Diptera |
| 353     | (304/313)                              | 97.1                            | MT123351 Calonectria ilicica / Nectriaceae / Hypocreales |
| 28      | (303/313)                              | 96.8                            | MK774334 Drunella ishiyamana / Ephemerellidae / Ephemeroptera |
| 200     | (303/313)                              | 96.8                            | JQ655116 Baetis sp. OPU_B5_B2011-494 / Baeitidae / Ephemeroptera |
| 117     | (302/313)                              | 96.5                            | LC329102 Eukiefferiella chuzeoctava / Chironomidae / Diptera |
| 186     | (302/313)                              | 96.5                            | LC329204 Polyplegium parvicumen / Chironomidae / Diptera |
| 54      | (302/314)                              | 96.2                            | LC462319 Nilotanytarsus dubius / Chironomidae / Diptera |
| 33      | (301/313)                              | 96.2                            | MH260779 Drunella sp. OPU_B5_D2016-90 / Ephemerellidae / Ephemeroptera |
| 47      | (301/313)                              | 96.2                            | MK774379 Serratella setigera / Ephemerellidae / Ephemeroptera |
| 104     | (301/313)                              | 96.2                            | LC329301 Tvetenia tamaflava / Chironomidae / Diptera |
| 167     | (301/313)                              | 96.2                            | AM398700 Micropsectra kurobemaculata / Chironomidae / Diptera |
| 248     | (301/313)                              | 96.2                            | KY633405 Slavina appendiculata / Naididae / Haplotaxida Oligochaeta |

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Table 1 (continued)

| GMYC ID | (Number of identical sites/query size) | Similarity scores (% identity) | INSD accession number | Organism name/ Family/ Order | Remark on taxon |
|---------|---------------------------------------|---------------------------------|-----------------------|-----------------------------|----------------|
| 128     | (300/312)                             | 96.2                            | LC329156 Parametriocephalus stylius / Chironomidae / Diptera     | Oligochaeta             |
| 252     | (299/313)                             | 95.5                            | AF534855 Pristina osborni / Naididae / Haplotaxida              |
| 71      | (298/313)                             | 95.2                            | MN961334 Neopera sp. OPU BS 2019-094-DB-PL / Perlidae / Plecoptera |
| 87      | (298/313)                             | 95.2                            | LC462305 Corynoneura kibunelata / Chironomidae / Diptera       |
| 214     | (298/313)                             | 95.2                            | KF563015 Baetia japonica / Baetidae / Ephemeroptera           |
| 233     | (296/311)                             | 95.2                            | KX107043 Rhyacophila brevicephala / Rhyacophilidae / Trichoptera |
| 57      | (297/313)                             | 94.9                            | MK774371 Oyamia sp. OPU_BS_2017-315-YS-PL / Perlidae / Plecoptera |
| 184     | (297/313)                             | 94.9                            | LC329139 Microtendipes tamaogouti / Chironomidae / Diptera     |
| 48      | (296/313)                             | 94.6                            | MK774379 Serratella setigera / Ephemereellidae / Ephemeroptera |
| 211     | (296/313)                             | 94.6                            | KP970709 Acentrella gnom / Baetidae / Ephemeroptera            |
| 122     | (294/311)                             | 94.5                            | LC462337 Orthocladius tamarutilus / Chironomidae / Diptera     |
| 142     | (294/312)                             | 94.2                            | KX946556 Tabanus thoracinus / Tabanidae / Diptera              |
| 58      | (294/313)                             | 93.9                            | MK774371 Oyamia sp. OPU_BS_2017-315-YS-PL / Perlidae / Plecoptera |
| 205     | (277/295)                             | 93.9                            | MH823348 Acentrella sibirica / Baetidae / Ephemeroptera       |
| 339     | (293/313)                             | 93.6                            | KR055655 Pseudogymnoascus pannorum / Pseudeurotiaceae / fungus |
| 361     | (293/313)                             | 93.6                            | MK468491 Ascochyta pisi / Didymellaceae / Pleosporales / fungus |
| 245     | (290/310)                             | 93.5                            | LN810254 Piquetiella blanchi / Naididae / Haplotaxida / Oligochaeta |
| 341     | (293/314)                             | 93.3                            | KR055655 Pseudogymnoascus pannorum / Pseudeurotiaceae / fungus |
| 61      | (292/313)                             | 93.3                            | AB770139 Oyamia lugubris / Perlidae / Plecoptera              |
| 72      | (292/313)                             | 93.3                            | MN961334 Neopera sp. OPU BS 2019-094-DB-PL / Perlidae / Plecoptera |
| 146     | (292/313)                             | 93.3                            | MG089252 Hexatoma sp. I13-26-02 / Limonidae / Diptera         |
| 218     | (291/312)                             | 93.3                            | HQ938480 Zaitzevia sp. BOLD:AAN5764 / Elmidae / Coleoptera    |
| 88      | (288/309)                             | 93.2                            | KM928316 Chironomidae sp. BOLD:AAN5033 / Chironomidae / Diptera |
| 344     | (267/287)                             | 93.0                            | KX450332 Glarea lozoyensis / Helotiaceae / Helotiales / fungus |
| 143     | (291/313)                             | 93.0                            | MK396368 Tabanus thoracinus / Tabanidae / Diptera              |
| 145     | (290/312)                             | 92.9                            | MG089252 Hexatoma sp. I13-26-02 / Limonidae / Diptera         |
| 132     | (289/311)                             | 92.9                            | AY251508 Simulium grossifilum / Simuliidae / Diptera           |
| 106     | (288/310)                             | 92.9                            | MF458772 Orthocladiinae sp. BAP34 / Chironomidae / Diptera     |
| 80      | (289/313)                             | 92.7                            | MK774371 Oyamia sp. OPU_BS_2017-315-YS-PL / Perlidae / Plecoptera |
| 141     | (290/313)                             | 92.7                            | DQ983533 Tabanus aegrotus / Tabanidae / Diptera                 |
| 124     | (286/309)                             | 92.6                            | MN680434 Pseudokiefferiella sp. BOLD:AAL9436 / Chironomidae / Diptera |
| 40      | (289/313)                             | 92.3                            | LC461324 Teloganopsis puncitsetae / Ephemereellidae / Ephemeroptera |
| 114     | (289/313)                             | 92.3                            | JF287767 Potthastia gaedii / Chironomidae / Diptera           |
| 116     | (289/313)                             | 92.3                            | HQ941600 Orthocladius rivulorum / Chironomidae / Diptera       |
| 144     | (288/313)                             | 92.0                            | KC592633 Scioniini gen. NZ sp. llerda / Tabanidae / Diptera     |
| 102     | (287/312)                             | 92.0                            | MT047747 Diamesa hyperborea / Chironomidae / Diptera           |
| 360     | (287/312)                             | 92.0                            | KM382246 Shiria bambusicola / Shiriaclaeae / Pleosporales / fungus |
| 227     | (285/310)                             | 91.9                            | MT230864 Parachauliodes continentalis / Corydalidae / Megaloptera |

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Table 1 (continued)

| GMYC ID | (Number of identical sites/query size) | Similarity scores (% identity) | INSD accession number | Organism name/ Family/ Order | Remark on taxon |
|---------|--------------------------------------|-------------------------------|-----------------------|-------------------------------|-----------------|
| 134     | (283/308)                            | 91.9                          | KP252587 Simulium gonzalezi / Simuliidae / Diptera |
| 7       | (287/313)                            | 91.7                          | MK774358 Rhithrogena tetrapunctigera / Heptageniidae / Ephemeroptera |
| 60      | (287/313)                            | 91.7                          | AB770139 Oyamia lugubris / Perlidae / Plecoptera |
| 100     | (287/313)                            | 91.7                          | KJ082995 Apsiphortica lini / Drosophilidae / Diptera |
| 125     | (286/312)                            | 91.7                          | MG301788 Orthocladiinae sp. BIOUG23941-E02 / Chironomidae / Diptera |
| 109     | (284/310)                            | 91.6                          | KX779818 Culex gnomatos / Culicidae / Diptera |
| 170     | (286/313)                            | 91.4                          | KT613675 Tanytarsus sp. 7xl / Chironomidae / Diptera |
| 99      | (283/310)                            | 91.3                          | LC329164C / Chironomidae / Diptera |
| 355     | (271/297)                            | 91.2                          | MK674497 Podosphaera xanthii / Erysisphaeae / Erisiphales |
| 271     | (279/306)                            | 91.2                          | MN673973 Sperchon glandulosus / Sperchontidae / Trombidiiformes |
| 2       | (285/313)                            | 91.1                          | MK774405 Rhithrogena japonica / Heptageniidae / Ephemeroptera |
| 64      | (285/313)                            | 91.1                          | AB770139 Oyamia lugubris / Perlidae / Plecoptera |
| 65      | (285/313)                            | 91.1                          | AB770139 Oyamia lugubris / Perlidae / Plecoptera |
| 340     | (284/312)                            | 91.0                          | EU883400 Tetracladium apiense / Helotiales |
| 115     | (281/309)                            | 90.9                          | KC750387 Cricotopus sp. 1 MEC-2013 / Chironomidae / Diptera |
| 342     | (279/307)                            | 90.9                          | KY318514 Pseudogymnoascus destructans / Pseudoeurotiaceae / Acariformes |
| 93      | (286/315)                            | 90.8                          | KR659696 Corynoneura sp. BOLD-2016 / Chironomidae / Diptera |
| 121     | (285/314)                            | 90.8                          | JX887678 Leucophenga saigusai / Drosophilidae / Diptera |
| 334     | (284/313)                            | 90.7                          | GU070900 invertebrate environmental sample / unspecified |
| 173     | (283/312)                            | 90.7                          | LC329269 Stempellinella tamaseptima / Chironomidae / Diptera |
| 228     | (283/312)                            | 90.7                          | KX294695 Rhyacophila lata / Rhyacophilidae / Trichoptera |
| 148     | (282/311)                            | 90.7                          | KX771129 Drosophila nikananu / Drosophilidae / Diptera |
| 243     | (282/311)                            | 90.7                          | GQ355369 Nais elinguis / Naididae / Haplotaxida |
| 154     | (272/300)                            | 90.7                          | KT116489 Gymnometriocnemus brunalis / Chironomidae / Diptera |
| 110     | (281/310)                            | 90.6                          | KF839967 Simulium callidum / Simuliidae / Diptera |
| 130     | (280/309)                            | 90.6                          | KP997234 Leucophenga sp. 7 HWC-2015 / Drosophilidae / Diptera |
| 139     | (267/295)                            | 90.5                          | JF871911 Docosia sp. BOLD:AAP4732 / Mycetophilidae / Diptera |
| 120     | (283/313)                            | 90.4                          | LC582930 Chironomidae sp. 11 NK-2020 / Chironomidae / Diptera |
| 352     | (283/313)                            | 90.4                          | JN574872 Calonectria colhounii / Nectriaceae / Hypocreales fungus |
| 101     | (282/312)                            | 90.4                          | MG308407 Gymnometriocnemus sp. BIOUG01460-H10 / Chironomidae / Diptera |
| 105     | (282/312)                            | 90.4                          | KY841662 Chironomidae sp. BIOUG02204-H08 / Chironomidae / Diptera |
| 94      | (281/311)                            | 90.4                          | MF825573 Chironomidae sp. BIOUG02749-B02 / Chironomidae / Diptera |
| 111     | (281/311)                            | 90.4                          | MG144554 Chironomidae sp. BIOUG27558-D07 / Chironomidae / Diptera |
| 140     | (280/310)                            | 90.3                          | JN887059 Cricotopus bimaculatus / Chironomidae / Diptera |
| 149     | (273/303)                            | 90.1                          | KY833856 Megaselia sp. BIOUG2394-D11 / Phoridae / Diptera |
| 67      | (282/313)                            | 90.1                          | MN344567 Kaminuria tibialis / Perlidae / Plecoptera |
| 147     | (281/312)                            | 90.1                          | MW301815 Pilaria sp. ZS10 / Limoniidae / Diptera |
| 155     | (275/306)                            | 89.9                          | LC057190 Drosophila trilutea / Drosophilidae / Diptera |
| 343     | (281/313)                            | 89.8                          | EU678468 Leohumicola sp. DAOM 239516 / fungus |
| 162     | (280/312)                            | 89.7                          | LC329265 Rheotanytarsus tamaquintus / Chironomidae / Diptera |

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Table 1 (continued)

| GMYC ID | (Number of identical sites/query size) | Similarity scores (% identity) | INSD accession number | Organism name/ Family/ Order | Remark on taxon |
|---------|----------------------------------------|-------------------------------|-----------------------|-------------------------------|-----------------|
| 269     | (280/312)                              | 89.7                          | MN362995 BIOUG15123-B05 / Lebertiidae / Trombidiformes | Acariformes |
| 273     | (280/312)                              | 89.7                          | MF744731 BIOUG18156-B10 / Hydrophantiidae / Trombidiformes | Acariformes |
| 138     | (279/311)                              | 89.7                          | MG301788 BIOUG23941-E02 / Chironomidae / Diptera | |
| 165     | (279/311)                              | 89.7                          | MF835529 BIOUG21146-F12 / Tachinidae / Diptera | |
| 350     | (279/311)                              | 89.7                          | MN593345 Arthrocladium fulminans / Trichomeriaceae / Chaetothyriales | |
| 158     | (278/310)                              | 89.7                          | KC263146 Antocha ss. SS-2012 / Limoniidae / Diptera | |
| 220     | (269/300)                              | 89.7                          | MF881631 Drosophilinae sp. BIOUG24085-F09 / Drosophilidae / Diptera | |
| 239     | (277/309)                              | 89.6                          | JQ907722 Goeracea genota / Goeridae / Trichoptera | |
| 151     | (274/306)                              | 89.5                          | JN285990 Zavrelinia sp. 1ES / Chironomidae / Diptera | |
| 89      | (280/313)                              | 89.5                          | LC462308 Corynoneura lobata / Chironomidae / Diptera | |
| 112     | (280/313)                              | 89.5                          | MG300230 Tanytarsinae sp. BIOUG21805-B09 / Chironomidae / Diptera | |
| 150     | (280/313)                              | 89.5                          | MF476250 Simulium aratum / Simuliidae / Diptera | |
| 262     | (278/311)                              | 89.4                          | MG895845 Sperchon glandulosus / Sperchontidae / Trombidiformes | Acariformes |
| 51      | (261/292)                              | 89.4                          | MG516460 Chromacys magnifica / Oligoneuriidae / Ephemeroptera | |
| 318     | (269/301)                              | 89.4                          | JN418688 Pinnularia sp. 9 CS-2011 / Pinnulariaceae / Naviculales | alga |
| 108     | (280/314)                              | 89.2                          | KR457791 Orthocladius sp. BOLD-2016 / Chironomidae / Diptera | |
| 153     | (277/311)                              | 89.1                          | KR436830 Nanocladius dichromus / Chironomidae / Diptera | |
| 175     | (267/300)                              | 89.0                          | HM385606 Tanytarsus sp. TTDW1048-09 / Chironomidae / Diptera | |
| 300     | (273/307)                              | 88.9                          | FN601011 Ptochoecetis africana / Leptoceridae / Trichoptera | |
| 172     | (278/313)                              | 88.8                          | LC495094 Benthalia sp. HM-2012 / Chironomidae / Diptera | |
| 78      | (277/312)                              | 88.8                          | MK774407 Paragnetina sp. OPU_BS_2018-050-TM-PL / Perilidae / Plecoptera | |
| 156     | (277/312)                              | 88.8                          | FM998448 Cheumatopsyche persica / Hydropsychidae / Trichoptera | |
| 274     | (277/312)                              | 88.8                          | MW369218 Sperchonopsis aff. verrucosa VZ19076 / Sperchontidae / Trombidiformes | Acariformes |
| 226     | (276/311)                              | 88.7                          | JK448423 Parasyctopus exsulans / Curculionidae / Coleoptera | Parasyctopus intrusus (Kôno, 1948) |
| 247     | (273/308)                              | 88.6                          | JQ519820 Chaetogaster diastrophus / Naididae / Haplotaxida | Oligochaeta |
| 270     | (271/306)                              | 88.6                          | MF458740 Lebertia porosa / Lebertiidae / Trombidiformes | Acariformes |
| 249     | (270/305)                              | 88.5                          | GQ355367 Chaetogaster diastrophus / Naididae / Haplotaxida | Oligochaeta |
| 83      | (277/313)                              | 88.5                          | LC462302 Corynoneura tenuistyla / Chironomidae / Diptera | |
| 163     | (277/313)                              | 88.5                          | KT613493 Tanytarsus tamadodecimus / Chironomidae / Diptera | |
| 178     | (277/313)                              | 88.5                          | KC750520 Riethia stictoptera / Chironomidae / Diptera | |
| 268     | (276/312)                              | 88.5                          | MW369264 Torrenticola aff. amplexa VZ19145 / Torrenticolidae / Trombidiformes | Acariformes |
| 84      | (268/303)                              | 88.4                          | KF489833 Corynoneura mediaspicula / Chironomidae / Diptera | |
| 193     | (260/294)                              | 88.4                          | MT262583 Simulium sp. BPD1 / Simuliidae / Diptera | |
| 330     | (274/310)                              | 88.4                          | KC791166 Neoporphyrha haitanensis / Bangiaceae / Bangiales red alga | |

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| GMYC ID | (Number of identical sites/query size) | Similarity scores (% identity) | INSD accession number | Organism name/ Family/ Order | Remark on taxon |
|---------|---------------------------------------|--------------------------------|-----------------------|-----------------------------|----------------|
| 169     | (278/315)                             | 88.3                           | JF287884              | BOLD:AAH3855 / Chironomidae/Diptera |
| 346     | (270/306)                             | 88.2                           | KY318514              | Pseudogymnoascus destructans/Pseudoeurotiaceae |
| 24      | (262/297)                             | 88.2                           | MH823334              | Propeorus nipponicus / Heptageniidae / Ephemeroptera |
| 176     | (276/313)                             | 88.2                           | MN150323              | Chironomidae / Diptera |
| 267     | (276/313)                             | 88.2                           | KJ709343              | Arrenurus sp. BOLD:ACH9257 / Arrenuridae / Trichobdiformes |
| 348     | (275/312)                             | 88.1                           | KR704425              | Verticillum nonalfalvae / Plectosphaerellaceae / Glomerellales |
| 229     | (267/303)                             | 88.1                           | GU711742              | Hiralosyphce sp. XZ CN1 / Rhycophilidae / Trichoptera |
| 49      | (270/307)                             | 87.9                           | MK403745              | Ablabesmyia monilis / Chironomidae / Diptera |
| 63      | (276/314)                             | 87.9                           | AB770139              | Oyamia lugubris / Perlidae / Plecoptera |
| 152     | (268/305)                             | 87.9                           | KY861853              | Dictenidia leigongshanensis / Tipulidae / Diptera |
| 80      | (275/313)                             | 87.9                           | MH840672              | Sweltsa borealis / Chloroperlidae / Plecoptera |
| 174     | (266/303)                             | 87.9                           | KP462171              | Chironomidae sp. WHW-2015 / Chironomidae / Diptera |
| 349     | (263/300)                             | 87.7                           | MN593345              | Arthrocladium fulminans / Trichomeriaceae / Chaetothyriales |
| 282     | (270/308)                             | 87.7                           | HM377216              | Hydraphantes sp. KOWMC070-09 / Hydraphantidae / Trichobdiformes |
| 52      | (268/306)                             | 87.6                           | LC329151              | Orthocladius kanii / Chironomidae / Diptera |
| 272     | (275/314)                             | 87.6                           | MW369201              | Protzia caucasica / Hydraphantidae / Trichobdiformes |
| 356     | (274/313)                             | 87.5                           | KU168424              | Cairneyella variabilis / Helotiales |
| 192     | (273/312)                             | 87.5                           | KU373672              | Ceratopogon sp. BOLD:AC11916 / Ceratopogonidae / Diptera |
| 221     | (272/311)                             | 87.5                           | HQ938470              | Ordobrevia nubifera / Elmidae / Coleoptera |
| 265     | (265/303)                             | 87.5                           | KU243801              | Testudacarus harrisii / Tortricidae / Trichobdiformes |
| 219     | (270/309)                             | 87.4                           | KT818884              | Hydora sp. EJD-2015 / Elmidae / Coleoptera |
| 195     | (256/293)                             | 87.4                           | KP252653              | Simulium travisi / Simuliidae / Diptera |
| 345     | (268/307)                             | 87.3                           | KX257489              | Cladophilaphora bantiana / Herpotrichiellaceae / Chaetothyriales |
| 62      | (274/314)                             | 87.3                           | AB770139              | Oyamia lugubris / Perlidae / Plecoptera |
| 191     | (273/313)                             | 87.2                           | JF872386              | Ceratopogon sp. BOLD:AG6465 / Ceratopogonidae / Diptera |
| 359     | (266/305)                             | 87.2                           | MK637641              | Sydowia polyspora / Dithioracaeae / Dithidales/ fungus |
| 157     | (273/314)                             | 86.9                           | EU493666              | Drosophila nigella / Drosophilidae / Diptera |
| 263     | (272/313)                             | 86.9                           | HQ938536              | Sperchon sp. BOLD:AN9213 / Sperchontidae / Trichobdiformes |
| 333     | (269/310)                             | 86.8                           | GU070501              | invertebrate environmental sample / unspecified |
| 232     | (268/309)                             | 86.7                           | MG511784              | Rhyacophilidae sp. 10BEEPT-0215 / Rhyacophilidae / Trichoptera |
| 13      | (273/315)                             | 86.7                           | MH844244              | Anopheles braziliensis / Culicinae / Diptera |
| 303     | (265/306)                             | 86.6                           | JN304924              | Chrysauginae sp. BOLD:AAK575 / Pyralidae / Lepidoptera |
| 50      | (258/298)                             | 86.6                           | MF668536              | Siphlenigma janae / Siphleagnatidiae / Ephemeroptera |
| 14      | (270/312)                             | 86.5                           | KP970706              | Rhithrogena tetrapunctigera / Heptageniidae / Ephemeroptera |
| 293     | (249/288)                             | 86.5                           | MN674851              | Hydraphantidae sp. BOLD:AD50542 / Hydraphantidae / Trichobdiformes |
| 190     | (270/313)                             | 86.3                           | MF105765              | Culicoides longipennis / Ceratopogonidae / Diptera |

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| GMYC ID | (Number of identical sites/query size) | Similarity scores (% identity) | INSD accession number | Organism name/ Family/ Order | Remark on taxon |
|---------|--------------------------------------|-------------------------------|----------------------|-------------------------------|-----------------|
| 68      | (263/306)                            | 85.9                          | KR665902 Drosophilidae/Diptera                  |                  |                 |
| 285     | (269/313)                            | 85.9                          | MW369236 Parathysa dirempta/Hydrachnidida/Tremblionformes |                 | Acariformes     |
| 347     | (268/312)                            | 85.9                          | MN657181 Cladosporium sphaerospermum/Clausporiales |                 | fungus          |
| 278     | (263/307)                            | 85.7                          | MG319031 Sperchontidae sp.BIOUG22338-D12/ Sperchontidae/Tromblionformes |                 | Acariformes     |
| 332     | (268/313)                            | 85.6                          | MT228925 Vexillifera sp. AKu-2020a/ Vexilliferae/Dactylpodida |               | Amoebozoa       |
| 284     | (265/310)                            | 85.5                          | MN364233 Parathysa sp. BOLD:AAM7957/Hydrachnidia/Tromblionformes |               | Acariformes     |
| 358     | (265/310)                            | 85.5                          | KX011536 Zasmidium cellare/Mycosphaerellae/Mycosphaerellales |               | fungus          |
| 5       | (264/309)                            | 85.4                          | KI675257 Cinygmula sp. JMW3/Heptageniida/Heptagenidida/Ephemeroptera |               |                 |
| 82      | (266/312)                            | 85.3                          | KX576477 Taenia nema atlanticum/ Taeioptrygidae/Plecoptera |               |                 |
| 251     | (258/303)                            | 85.1                          | GQ355374 Pristina aequi seta/Naididae/Haplotaxidea/ |               | Oligochaeta     |
| 283     | (263/309)                            | 85.1                          | KC263080 Sperchon sp. SS-2012/Sperchontidae/Tromblionformes |          | Acariformes     |
| 4       | (267/314)                            | 85.0                          | MN961316 Heptageniidae sp. OPB BS 2019-017-DB-EP/Heptageniida/Ephemeroptera |           |                 |
| 208     | (260/306)                            | 85.0                          | KR489256 Melanophilalma helvola/Latriidiidae/Coleoptera |           |                 |
| 280     | (262/309)                            | 84.8                          | MG835761 Axonopsis sp. 1BHL041917/Aturida/Tremblionformes |           | Acariformes     |
| 254     | (261/308)                            | 84.7                          | LN999074 Enchytraeidae sp. 1 RV-2016/Enchytraeida/Enchytraeida |         | Oligochaeta     |
| 207     | (266/314)                            | 84.7                          | MN640425 Cloeon perkinsi/Baetidae/Ephemeroptera |            |                 |
| 206     | (265/313)                            | 84.7                          | JQ662499 Baetodes sp. JM1/Baetidae/Ephemeroptera |            |                 |
| 259     | (265/313)                            | 84.7                          | KP845499 Harpacticoida sp. BOLD:ACM2620/Harpacticoida |           |                 |
| 222     | (257/304)                            | 84.5                          | FJ81996 Acanaca sp. 5 MTM-2009/Hyphilidae/Coleoptera |           |                 |
| 264     | (267/316)                            | 84.5                          | MF46983 Sperchontidae sp. BIOUG18716-A05/Sperchontidae/Tromblionformes |           | Acariformes     |
| 201     | (264/313)                            | 84.3                          | MT830952 Labiobates sabordoi/Baetidae/Ephemeroptera |           |                 |
| 253     | (260/309)                            | 84.1                          | GU014012 Megascoleidae sp. DPEW46886/Megascoleidae/Crassiclitellata |          | Opisthoptora    |
| 363     | (233/277)                            | 84.1                          | HQ923626 Phruxocomes sp. ANIC7/Geometricida/Lepidoptera |         | moth            |
| 189     | (254/302)                            | 84.1                          | KX453756 Liponeura cordata/Blepharicerida/Diptera |            |                 |
| 311     | (259/308)                            | 84.1                          | MN356242 Oribatella sp. BOLD:AIL8123/Oribellitida/Sarcopostiformes |         |                 |
| 6       | (253/301)                            | 84.1                          | HQ261141 Paraleptophlebia sp. AMI 1/Leptophyslebiida/Ephemeroptera |         |                 |
| 321     | (252/300)                            | 84.0                          | MT222521 Globisporangium spinosum/Pythiaceae/Pythiales |         | fungus          |
| 204     | (262/312)                            | 84.0                          | KF636303 Baetis thermicus/Baetida/Ephemeroptera |            |                 |
| 367     | (262/312)                            | 84.0                          | MW369294 Atractides tener/Hydrobatida/Tromblionformes |           |                 |
| 73      | (261/311)                            | 83.9                          | MK132423 Nemoura sp. 3 MG-2018/Nemourida/Plecoptera |           |                 |
| 203     | (261/311)                            | 83.9                          | MH841573 Baetis bicaudatus/Baetida/Ephemeroptera |           |                 |
| 317     | (259/285)                            | 83.9                          | E165193 Selliaphora pupula/Selliaphoraceae/Naviculales/ |        | alga            |
| 319     | (258/308)                            | 83.8                          | M97422 Nitzchia alba/Bacillariae/Bacillariales |          |                 |
| 290     | (262/313)                            | 83.7                          | LR827697 Agraylea multipunctata/Hydroptilidae/Trichoptera |          |                 |

(continued on next page)
| GMYC ID | (Number of identical sites/query size) | Similarity scores (% identity) | INSD accession number | Organism name/ Family/ Order | Remark on taxon |
|---------|--------------------------------------|-------------------------------|---------------------|-----------------------------|----------------|
| 289     | (241/288)                            | 83.7                          | MW369278 Lebertia porosa / Lebertiidae / Trombidiformes | Acariformes                  |
| 69      | (256/306)                            | 83.7                          | KR665902 Drosophila paramelanica / Drosophilidae / Diptera |                                      |
| 304     | (261/312)                            | 83.7                          | KZ296397 Hydroptilidae sp. TVTRI0057 / Hydroptilidae / Trichoptera |                                      |
| 275     | (255/305)                            | 83.6                          | MN359216 Mideopsis sp. BOLD:AAL8123 / Mideopsisidae / Trombidiformes | Acariformes                  |
| 357     | (260/311)                            | 83.6                          | EU678467 Myxotrichium deflexum / Myxotrichiaceae / Hymenoptera | fungus                      |
| 324     | (137/164)                            | 83.5                          | KR879316 Pholetesor sp. BOLD-2016 / Braconidae / Hymenoptera | wag                           |
| 331     | (246/295)                            | 83.4                          | KR665197 M ycophilidae sp. BOLD-2016 / M ycophilidae / Diptera |                                      |
| 32      | (256/307)                            | 83.4                          | JQ661610 Ephemerella dorothea / Ephemerellidae / Ephemeroptera |                                      |
| 256     | (264/317)                            | 83.3                          | KY633394 Branchi diritis hortensis / Naididae / Haplotaxida | Oligochaeta                  |
| 217     | (259/311)                            | 83.3                          | KR561360 Salidiae sp. BOLD-2016 / Salidiae / Hemiptera | shore bug                   |
| 281     | (259/311)                            | 83.3                          | JX838402 Hydr phantes sp. BOLD:AAF4149 / Hydroptilidae / Trombidiformes | Acariformes                  |
| 320     | (251/302)                            | 83.1                          | KF768027 Dimi eragma acutum / Plagiogrammaceae / Triceratiales | alga                        |
| 327     | (187/225)                            | 83.1                          | MT925539 Protancylus adhaerens / Protancylidae / Hyphphila | Gastrapoda                   |
| 23      | (260/313)                            | 83.1                          | KM570892 Dolichopodidae sp. BOLD:AAG9626 / Dolichopodidae / Diptera |                                      |
| 198     | (259/312)                            | 83.0                          | HG935109 Cloeon cf. smaelei GL30 / Baetidae / Ephemeroptera |                                      |
| 276     | (259/312)                            | 83.0                          | MG316728 Mideopsis sp. BIOUG23251-F06 / Mideopsisidae / Trombidiformes | Acariformes                  |
| 286     | (259/312)                            | 83.0                          | MG449836 Pionidae sp. BIOUG30216_F02 / Pionidae / Trombidiformes | Acariformes                  |
| 279     | (261/315)                            | 82.9                          | MW369209 Lebertia aff. inaequalis VZ19067 / Lebertiidae / Trombidiformes | Acariformes                  |
| 335     | (251/303)                            | 82.8                          | MH124198 Acanthamoeba sp. / Acanthamoebidae / Longamoebia | Amoebozoa                    |
| 291     | (260/314)                            | 82.8                          | HQ563462 Micropterix schaefferi / Micropterigidae / Lepidoptera | moth                         |
| 36      | (259/313)                            | 82.7                          | KM954873 Dolichopodidae sp. BOLD:ACB1149 / Dolichopodidae / Diptera |                                      |
| 202     | (258/312)                            | 82.7                          | KR134645 Baetodes sp. gmycM20 / Baetidae / Ephemeroptera |                                      |
| 292     | (258/312)                            | 82.7                          | MG311338 Lebertia sp. BIOUG25361-C07 / Lebertiidae / Trombidiformes | Acariformes                  |
| 316     | (243/294)                            | 82.7                          | MH681084 Pinnularia macilenta / Pinnulariaceae / Naviculales | alga                        |
| 277     | (238/288)                            | 82.6                          | MG315676 Hydrodroma sp. BIOUG23251-F10 / Hydrodromidae / Trombidiformes | Acariformes                  |
| 266     | (257/311)                            | 82.6                          | JN018109 Torrenticola amplexa / Torrenticolidae / Trombidiformes | Acariformes                  |
| 241     | (244/296)                            | 82.4                          | KX842664 Platysticta serendibica / Platystictidae / Odonata |                                      |
| 312     | (244/296)                            | 82.4                          | MN674518 Oppiidae sp. BOLD:AAL7979 / Oppiidae / Sarcopiformes | Acariformes                  |
| 288     | (229/278)                            | 82.4                          | KP966651 Penthaeleidae sp. Q080 / Penthaeleidae / Trombidiformes | Acariformes                  |
| 44      | (252/306)                            | 82.4                          | HE651331 Compsoneuria sp. 6 LV-2012 / Heptageniidae / Ephemeroptera |                                      |
| 43      | (261/317)                            | 82.3                          | KY262520 Serratella ignita / Ephemeroellidae / Ephemeroptera |                                      |
Table 1 (continued)

| GMYC ID | (Number of identical sites/query size) | Similarity scores (%) identity | INSD accession number | Organism name/ Family/ Order | Remark on taxon |
|---------|----------------------------------------|--------------------------------|-----------------------|-------------------------------|-----------------|
| 42      | (247/300)                              | 82.3                           | KJ964059 Melanophila acuminate / Buprestidae / Coleoptera       |                               |                 |
| 37      | (255/311)                              | 82.0                           | KX038172 Zephebia pionrnga / Leptopliidea / Ephemeroptera       |                               |                 |
| 366     | (112/137)                              | 81.8                           | KY698160 Cladius grandis / Tenthredinidae / Hymenoptera wasp |                               |                 |
| 260     | (250/307)                              | 81.4                           | MH976580 Eschizopera sp. n. aff. syltensis SR-2019 / Miraciidae / Harpacticoidea | wasp copepod |                 |
| 338     | (245/301)                              | 81.4                           | EU681393 Bachelotia antillarum / / alga                     |                               |                 |
| 75      | (244/300)                              | 81.3                           | KY428239 Hexachaeta eximia / Tephritidae / Diptera           |                               |                 |
| 328     | (230/283)                              | 81.3                           | MT380098 Megastigmus sp. 8 NHL-2020 / Megastigmidae / Hymenoptera | wasp coleoptera |                 |
| 35      | (245/302)                              | 81.1                           | MN961290 Ephemera strigata / Ephemeroptera / Ephemeroptera |                               |                 |
| 313     | (247/306)                              | 80.7                           | MN348626 Micronothus sp. BOLD:AA6084 / Trhypochthoniidae / Sarcoptiformes | Acariformes |                 |
| 258     | (244/303)                              | 80.5                           | MN745842 Ctenocalanus citer / Calanidae / Calanoida copepod |                               |                 |
| 325     | (225/281)                              | 80.1                           | KY492523 Stelligera rigida / Hemierasterellida / Tethyida sponge |                               |                 |
| 314     | (224/280)                              | 80.0                           | HQ948517 Elachista discina / Elachistidae / Lepidoptera moth |                               |                 |
| 326     | (238/299)                              | 79.6                           | KR245768 Phronia sp. BOLD:ACL1048 / Mycetophilidae / Diptera |                               |                 |
| 257     | (241/303)                              | 79.5                           | MG936104 Harpacticoidea sp. 11AlgonqNl0018 / Harpacticoidea copepod |                               |                 |
| 261     | (241/304)                              | 79.3                           | KM611754 Cyclopoida sp. BOLD:AA9785 / Cyclopoida copepod |                               |                 |
| 287     | (241/304)                              | 79.3                           | MG320138 Tiphis sp. 09PROBE-02368 / Pionidae / Trombidiiformes | Acariformes |                 |
| 309     | (241/307)                              | 78.5                           | MF917435 Suctobellidae sp. BIOUG20423-H02 / Suctobellidae / Sarcoptiformes | Acariformes |                 |
| 329     | (243/310)                              | 78.4                           | MK33920 Demospongiae sp. DGM-2019 / / sponge |                               |                 |
| 310     | (246/314)                              | 78.3                           | MN356725 Ceratoppia sp. BOLD:ADH191 / Ceratoppiidae / Sarcoptiformes | Acariformes |                 |
| 315     | (242/309)                              | 78.3                           | KX651129 Dendrobaena octaedra / Lumbricidae / Oligochaeta Crassicellitella |                               |                 |
| 368     | (217/279)                              | 77.8                           | KX281460 Stigmella sp. FicusauriculataVietnam / Nepticulidae / Lepidoptera | moth |                 |
| 364     | (232/299)                              | 77.6                           | HQ708994 Pythium vanterpooli / Pythiaceae / Pythiales fungus |                               |                 |
| 323     | (224/293)                              | 76.5                           | HM033152 Rhodomenia stenoglossa / Rhodomeniaceae / Rhodimiales red algea |                               |                 |
| 365     | (221/295)                              | 74.9                           | MN116495 Beybienkonus acuticercus / Corydiidae / Blattodea cockroach |                               |                 |
| 369     | (217/302)                              | 71.9                           | KY264161 Thaumameris zealandica / Mermithidae / Merithida nematoda |                               |                 |

* Species showing high similarity scores (>95%) but not recorded from Japan.

the DNA libraries was assessed by Fragment Analyzer with dsDNA 915 Reagent Kit (Agilent Technologies, Santa Clara, CA, USA). High-throughput parallel sequencing was performed by Miseq (Illumina, San Diego, CA, USA) capable of generating 2 × 300 base paired-end reads. The library construction (after the first PCR) and sequencing were outsourced to Seibutsu Giken Co. Ltd. (Sagamihara, Japan) (https://gikenbio.com/).

2.3. Operational Taxonomic Units

Among the raw sequence reads (472,697 reads in total of DRR311861-DRR311868), reads with quality scores less than 20 were discarded, and those of the 313 bp net length of paired sequences were sorted out. Valid sequences (227,467 reads) were collapsed into 41,010 groups with 100% identity, and the groups consisting of less than three reads were discarded. Neighbouring
Fig. 1. Species clusters estimated by GMYC. Each vertical bar indicates a cluster of OTUs or singleton assigned to single GMYC species. OTUs are numbered arbitrarily, and the numbers in parentheses after OTU names indicate the number of reads of OTUs. Numbers with dotted lines refer to GYMC IDs. Owing to the size of the phylogenetic tree, Fig. 1 is divided into 1a and 1b, both of which are linked at the node indicated by the closed circle (●).
groups with a single nucleotide difference were combined each other to form an OTU, and a sequence with the largest number of reads within the OTU was defined as the OTU representative sequence. A total of 888 OTUs were obtained and subjected to GMYC analysis. The R script used to produce OTUs and OTU representatives in FASTA format are deposited in Mendeley Data (DOI: 10.17632/pfhf4kt37s.1).

2.4. Estimation of GMYC species

A phylogenetic tree of 888 OTUs was reconstructed using Markov chain Monte Carlo (MCMC) methods by MrBayes 3.2.7a with the GTR+I+G molecular clock model. GMYC species boundaries were estimated using SPLITS package in R.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Operational taxonomic units of cox1 amplified from bulk community DNA samples in Takamigawa River, Nara, Japan (Original data) (Mendeley Data).
cox1 amplicon sequences of riverine metagenomes (Original data) (DDBJ (DNA Data Bank of Japan)).

CRediT Author Statement

Kei Wakimura: Investigation, Writing – original draft; Koji Inai: Software, Investigation; Kazumi Tanida: Resources, Writing – review & editing; Kozo Watanabe: Conceptualization,
Writing – review & editing, Funding acquisition; Mikio Kato: Project administration, Conceptualization, Resources, Writing – review & editing, Funding acquisition.

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