Biochemical Systematics and Ecology 61 (2015) 329–335

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Twenty-one new microsatellite markers for the ecologically important midge *Heterotrissocladius marcidus*, and their use in studies of alpine lakes

Katarína Goffová, Zuzana Čiamporová-Zat'ovičová*, Fedor Čiampor Jr.

Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, 845 06 Bratislava, Slovakia

ARTICLE INFO

Article history:
Received 17 March 2015
Received in revised form 29 June 2015
Accepted 30 June 2015
Available online 9 July 2015

Keywords:
Aquatic insects
Chironomidae
Nuclear DNA
SSR markers
454 pyrosequencing
Carpathians

ABSTRACT

Heterotrissocladius marcidus (Walker, 1856) (Diptera: Chironomidae) inhabits aquatic habitats across the Holarctic and Oriental regions, including remote alpine glacial lakes. It is thus a promising species for studies of population structure and genetic diversity in these highly vulnerable habitats. Here, 21 polymorphic microsatellites for *H. marcidus* are described, obtained using 454 GS-FLX pyrosequencing. The number of alleles per locus varied from 2 to 9 and no significant linkage disequilibrium was observed. To test applicability, 40 individuals were used from two lakes in the Tatra Mts (Western Carpathians). Observed and expected heterozygosity varied from 0 to 0.9 and 0.102 to 0.888, respectively. In both populations, three loci deviated significantly from Hardy–Weinberg equilibrium after Bonferroni correction, probably due to presence of null alleles or undetected biological processes. Application of microsatellites was tested on six alpine lake populations. Bayesian cluster analysis assigned individuals to lakes of their origin and revealed limited gene-flow between them. Five loci were successfully cross-amplified in the related midge *Macropelopia* sp. (Tanypodinae) and two in *Pseudodiamesa branickii* (Nowicki, 1873) (Diamesinae). The microsatellites described herein proved to be useful for genetic studies of alpine populations, and can provide important data for management and conservation of these threatened habitats.

1. Introduction

Alpine glacial lakes represent unique aquatic habitats, almost always inhabited by specific invertebrate assemblages adapted to severe, high-altitude conditions (Füreder, 1999; Füreder et al., 2006; Lencioni, 2004). Unfortunately, these lakes are susceptible to even minor environmental changes and a number of careless human activities over recent decades have had a dramatic effect, in particular on the fauna of these fragile habitats (Fjellheim et al., 2000; Skjelkvåle and Wright, 1998). These changes might subsequently be reflected in the genetic structure of resident populations, where they can be detected using appropriate molecular markers. However of previously published studies addressing genetic structure of alpine or montane aquatic invertebrates, most employ mitochondrial markers and focus almost exclusively on the fauna of streams

* Corresponding author.
E-mail address: zuzana.zatovicova@savba.sk (Z. Čiamporová-Zat'ovičová).

http://dx.doi.org/10.1016/j.bse.2015.06.037
0305-1978/

(e.g. Kubow et al., 2010; Pauls et al., 2009; Taubmann et al., 2011). Nuclear markers that could be used to study species inhabiting alpine lentic habitats or the evolution of their populations remain surprisingly scarce.

The non-biting midge, *Heterotrissocladius marcidus* (Walker, 1856) (Chironomidae: Orthocladiinae), is a promising candidate for such studies. Thought to be the most common chironomid species in subalpine and alpine lakes (Boggero et al., 2006; Füreder et al., 2006; Laville, 1971; Lods-Crozet et al., 2012; Sæther, 1975), including inlet and outlet zones (Hamerlík et al., 2006; Maiolini et al., 2006), it also makes up a large proportion of benthic macroinvertebrate assemblages in mountain lake districts across Europe. In addition, benthic larvae of H. marcidus also occur in cool and moderate temperature running-water habitats at a wide range of altitudes and in ponds, streams, rivers and springs, including cold lowland ones, from the eucrenal to the epirithral zone (Brabec et al., 2007; Orendt, 2000) across the Holarctic and Oriental regions (Sæther and Spies, 2013). Despite this extensive geographic range, individual *H. marcidus* populations tend to be small and isolated probably due to low mobility and specific microhabitat or feeding preferences of the species (Sæther, 1975). The patchy distribution might also be influenced by anthropogenic disturbance and so should not be considered wholly natural (Hamerlík and Bitušík, 2009).

Despite the evident ecological importance of *H. marcidus*, details of the species genetic structure and gene flow between its populations are rather poorly known. In fact, population genetic data about aquatic insects in general, and small ones such as chironomids in particular, are critically scarce. On one hand, midges are one of the largest groups of aquatic insects, with huge numbers of ecologically important indicator species (more than 1000 hits on "Chironomidae" in Web of Science since 2010), while on the other, previously published microsatellite data covers no more than four species, with no more than 12 loci used (Carew et al., 2013; Kaiser and Heckel, 2009; Nowak et al., 2006; Rasic et al., 2009).

In this study, the development of new polymorphic microsatellites for non-model midge *H. marcidus* using 454 pyrosequencing (Malausa et al., 2011) is reported. The *de novo* markers were tested for ease of use and applicability on lake populations from the High Tatra Mountains. The polymorphic markers were also cross-amplified for two additional chironomids, *Macropelopia* sp. (Tanypodinae) and *Pseudodiamesa branickii* (Nowicki, 1873) (Diamesinae), to test their possible applicability in different subfamilies of Chironomidae. The microsatellite markers described herein could provide valuable tool for small- and large-scale population genetic studies, and enhance monitoring activities and effective conservation management of aquatic habitats.

2. Material and methods

2.1. Sample collection

Sampling of chironomid larvae (Fig. 1c) was performed during the ice-free seasons of 2011–2013 at seven alpine lakes and ponds (Fig. 1b): specimens from the Okrúhle pleso were used for NGS, Zmrzlé pleso (Pop1), Zelené Kačacie pleso (Pop2), Stredné Kozie pleso (Pop3), Nižné Volie pliesko (Pop4), Nižné Wahlenbergovo pleso (Pop5), and Vyšné Zbojnícke pleso (Pop6) were used for testing application of developed microsatellites. The water bodies (1762–2105 m a.s.l.), were of glacial origin, located in four different valleys in the High Tatra Mountains, the highest part of the Western Carpathians (49°10′ N, 20°10′ E, Slovakia, Central Europe, Fig. 1a). Kick-samples of the littoral substratum were collected in hand-nets (300 μm mesh size) and retained material was fixed with ethanol in the field, then hand-sorted and identified to appropriate taxonomic level using a stereomicroscope and stored at −25 °C in 96% ethanol.

2.2. NGS, microsatellite development and testing

Next Generation Sequencing (NGS) was carried out using 15 *H. marcidus* larvae. The variability of microsatellite loci was evaluated using specimens of the two isolated populations (20 individuals per lake) from Zelené Kačacie pleso (Pop2) and Nižné Volie pliesko (Pop4). Samples from six lakes (Pop1–Pop6; 20 specimens per lake) were used for population clustering analysis, and cross-species amplification was carried out using 5 individuals of *Macropelopia* sp. (subfamily Tanypodinae) from Zelené Kačacie pleso and 5 individuals of *Pseudodiamesa branickii* (Nowicki, 1873) (Diamesinae) from Nižné Volie pliesko.

Genomic DNA from all specimens was isolated using a DNeasy Blood and Tissue extraction kit (Qiagen) according to the manufacturer's protocol, and stored at −25 °C at the Institute of Zoology SAS, Bratislava, Slovakia.

Because of the very small size of *H. marcidus* larvae, the total genomic DNA for NGS was isolated and pooled from 15 specimens. DNA was subject to 454 pyrosequencing at the GS-FLX LAB of Macrogen (Seoul, South Korea). 1/8 plate generated 104,299 reads (50.3 Mb) with an average length of 414.244 bp and 33% GC content.

Raw data (a single Fasta file containing all reads) were screened for di-, tri-, tetra- and pentanucleotide repeats with at least four repetitions using Msatcommander v0.8.2 (Faircloth, 2008). A total of 272 loci, suitable for primer design, were detected by Primer3 software (Rozen and Skaletsky, 2000). Total length of microsatellite repeat (max 350 bp) and number of repetitions (min 4) were the most important selection criteria. Sequences were aligned using Geneious Pro v6.0.5 (Kearse et al., 2012) to avoid repeated selection of the same loci. Finally, primer pairs were designed for 95 fragments 155–367 bp long (Supplementary data 1), and a short M13 sequence tail (CACGACGTTGTAAAACGAC) was added to the shorter fragment of each pair.