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Molecular data support the dispersal ability of the glacier tardigrade *Hypsibius klebelsbergi* Mihelčič, 1959 across the environmental barrier (Tardigrada)

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(with 4 figures)

Abstract

Two populations of the obligate glacier dweller, the tardigrade *Hypsibius klebelsbergi* Mihelčič, 1959, have been compared based on the mitochondrial COI gene fragment (DNA-barcode), a character hitherto unknown for this species. The animals originated from cryoconite holes on two separated glaciers located at different altitudes in the Ötztal Alps. The lack of divergence in the mitochondrial COI as well as in nuclear 18S and 28S rRNA gene sequences between these two populations indicates the presence of probably only one population on both glaciers separated by a mountain's ridge. Sequence data of the 18S rRNA gene are compared with such data already available for *H. klebelsbergi*.

K e y w o r d s: Tardigrada, *Hypsibius klebelsbergi*, populations, glaciers, dispersion, COI, DNA-barcode, 18S rRNA, 28S rRNA, the Ötztal Alps, Austria.

Introduction

The eutardigrade *Hypsibius klebelsbergi* Mihelčič, 1959 (the Hypsibiidae) represents the obligate glacier dweller (Dastych 2009, 2015) recorded so far only from several glaciers in the Austrian Central Alps. The species (Figs 1, 2) inhabits there the water-filled micro-caverns on the glacier surface, so-called cryoconite holes (*e.g.* Steinbock 1936, 1957, Dastych *et al.* 2003). Two other taxa, supposedly also obligate glacier inhabitants, *Hypsibius janetscheki* Ramazzotti, 1968 and *H. thaleri* Dastych, 2004, have been once only found on the glacier Nero in the Himalayas (Ramazzotti 1968, Janetschek 1990, Dastych 2004 a, b).

Hypsibius klebelsbergi has been recently re-described, information on its biology summarized and partly expanded (Dastych *et al*. 2003). A very dark pigmentation of the integument in the species (black or blackish-brown: Fig. 2), an unusual feature for eutardigrades, as well as the morphology and function of its osmoregulatory/excretory organs have been lately examined (Greven *et al*. 2005, Peltzer *at al*. 2007, respectively). Although several molecular characteristics of *H. klebelsbergi* are already known (Kiehl *et al*. 2007a, b, D'Haese *et al.* 2011, Prasath *et al.* 2012, 2013), no data on its

Fig. 1. *Hypsibius klebelsbergi* Mihelčič (the glacier Langtaler Ferner; SEM).

mitochondrial cytochrome *c* oxidase subunit I (COI) gene, which is used as DNA-barcode (Hebert *et al.* 2003) were available.

In this paper we analysed populations of *H. klebelsbergi* from two different glaciers based on the COI gene employed here for the first time in this taxon. Moreover, 18S rRNA and 28S rRNA sequences already known for the species (see Kiehl *et al*., *l.c.*) has been compared with the data obtained in this study. The discrete microhabitats (cryoconite holes) of *H. klebelsbergi* and a mountain's ridge separating both glaciers seem to suggest the limited gene flow between both populations that should be expressed in genetic divergence between them. On the other side the *H. klebelsbergi* is though to have a distinct dispersal ability due to producing anhydrobiotic tuns capable to propagate by wind (Kraus 1977).

Material and methods

The material originates from two glaciers in the Ötztal Alps located in the Gurgler Tal valley near Obergurgl (Nordtirol, Austrian Central Alps). They are:

1. The glacier Langtaler Ferner (in the Langtal valley: N 46° 47' 50.8'', E 11°00' 42.5'', sediment from cryoconite hole at the beginning of the glacier, ca 2660 m a.s.l., 4.09.2008, coll. H. Dastych;
2. The glacier Ramolferner (in the Ramolkamm range: N 46° 50' 25.6'', E 10° 58' 04.9'', sediment from cryoconite hole, 3175 m a.s.l., 10.09.2011, coll. H. Dastych.

Both localities on these glaciers are separated by ca 5.2 km in linear distance. Between them lies diagonally the mountain ridge Schwärzenkamm, a range elevated up to 3200 m a.s.l., forming partly a terrain barrier.

The samples of cryoconite sediment containing *H. klebelsbergi* have been collected by method described in Dastych *et al.* (2003) and initially processed in the laboratory of the Alpine Forschungsstelle Obergurgl. Living tardigrades with the sediment were brought to Hamburg and preserved in 100% ethyl alcohol. Five animals from each glacier have been selected for DNA extraction.

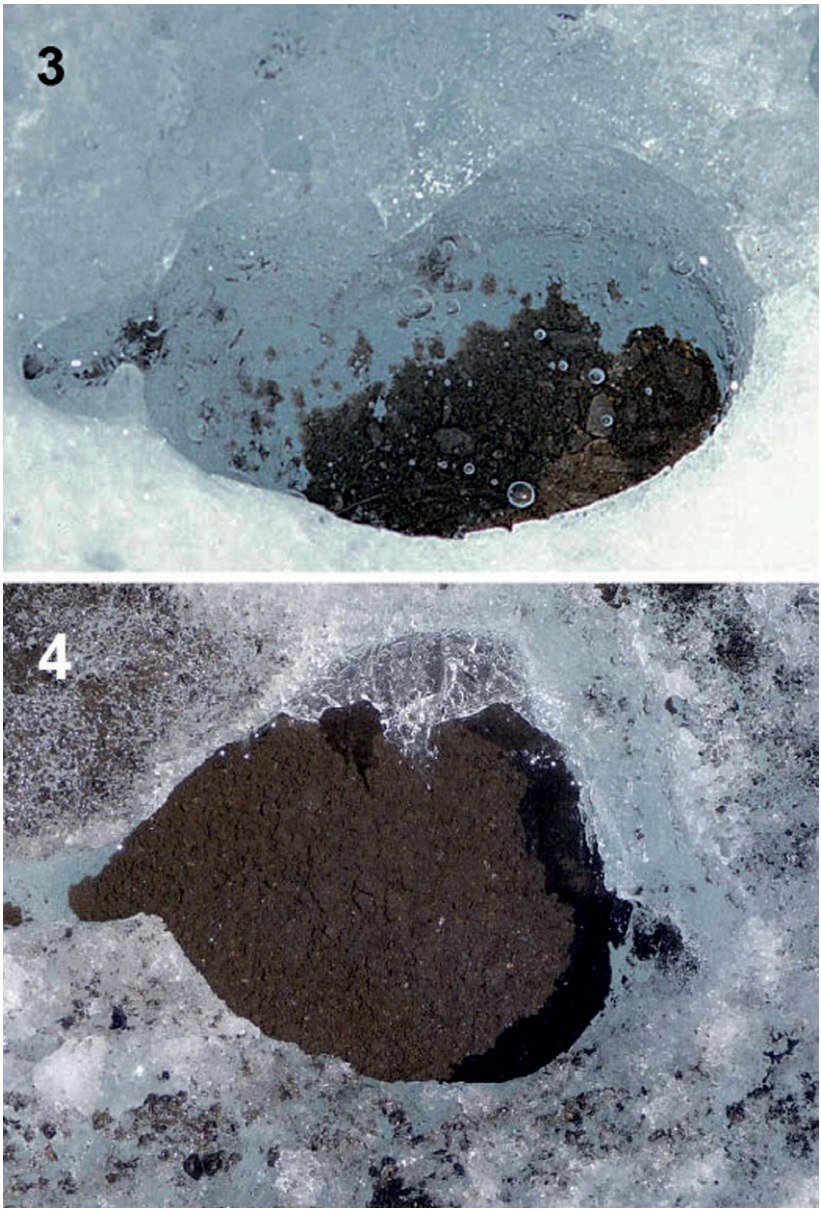
DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from individual specimens using a nondestructive method as previously described (Dabert *et al.* 2008, Dabert *et al.* 2013). For each analyzed species, five specimens were individually extracted and sequenced to check the sequence homogeneity. DNA barcode (*ca.* 650-bp region near the 5` terminus of the COI gene) was amplified by PCR using degenerated primers

bcdF01 (CATTTTCHACTAAYCATAARGATATTGG)
and bcdR04 (TATAAACYTCDGGATGNCCAAAAAA).

PCRs were carried out in 10 μl reaction volumes containing Type-it Microsatellite Kit (Qiagen), 0.5 μM each primer, and 4 μl of DNA template using a thermocycling profile of one cycle of 5 min at 95 °C followed by 35 steps of 30 sec at 95 °C, 1 min at 50 °C, 1 min at 72 °C, with a final step of 5 min at 72 °C. After amplification, the PCR reaction was two times diluted with water and 5 μl of the sample was analyzed by electrophoresis on a 1.5% agarose gel. Samples containing visible bands were

Fig. 2. *Hypsibius klebelsbergi* Mihelčič (the glacier Rotmoosferner; living, water-mounted animal; PHC).



Figs 3-4. Sediment in cryoconite holes: **3** - on the glacier Langtaler Ferner (note air-bubbles under thin ice layer after a night frost), **4** - on the glacier Ramolferner; the holes have 15-20 cm in diameter).

directly sequenced in both directions using 1–3 μl of the PCR reaction and 40 pmoles of primer. Sequencing was performed with BigDye Terminator v3.1 on an ABI Prism 3130XL Analyzer (Applied Biosystems). The18S and 28S rRNA genes were amplified and sequenced as previously described (see Dabert *et al.* 2013). Contigs were aligned and manually assembled in ChromasPro v. 1.32 (Technelysium Pty Ltd.) and GeneDoc v. 2.7.000 (Nicholas & Nicholas 1997). Sequences were deposited in GenBank (GenBank Accession nos. KT901827-KT901834). Pairwise distance calculations between sequences were computed using K2P distance model (Kimura 1980).

Results

We amplified and sequenced the COI gene fragment for five individual specimens representing each population. All COI sequences from specimens collected at the glacier Langtaler Ferner were identical, while small variation was observed in COI sequences found in specimens collected at the glacier Ramolferner. The nucleotide substitutions were C/T transitions in three nucleotide positions that resulted in four different haplotypes. The COI sequence found in specimens collected at the glacier Langtaler Ferner corresponded to the one of the COI haplotypes found in two specimens from the glacier Ramolferner. Intraspecific K2P divergence in COI sequences of *H. klebelsbergi* was 0.03%. No intraspecific variability in 18S (1730 bp) and 28S rRNA (3692 bp) gene fragments was detected. Comparison of our 18S rRNA sequence with one already known for the species (AM500648.1, see Kiehl *et al.*, *l.c.*) revealed an ATGC insertion in the sequence published previously. This insertion was found in a relatively conserved region of the gene and has never been observed in the other Tardigrada 18S rRNA sequences published so far.

Discussion

The lack of sequence differentiation between populations of *H. klebelsbergi* from both glaciers suggests that the gene flow occurred between both populations. In spite of discrete distribution of tardigrades in the glacier (separated cryconite holes) and a partial geographic barrier between glaciers (the Schwärzenkamm ridge), *H. klebelsbergi* disperses freely in both locations. The former observations concerning their possible distinct dispersal ability as anhydrobiotic tuns (Kraus 1977) is supported by our molecular data. These observations should be falsified by future investigations supported by much more extensive sampling in various locations and by applying more appropriate molecular markers (*e.g.* microsatellites).

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